

Claims

- [c1] 1. A method for transforming a monocotyledonous plant using an *Agrobacterium* -mediated process, comprising:
- (a) preculturing at least one immature embryo from a monocotyledonous plant in a first medium containing increased MS salts from between about 1.5 times standard to about 3 times standard and a concentration of picloram from between about 2.5 mg/L to about 5 mg/L for a period of time sufficient to form a precultured embryo;
 - (b) contacting the precultured embryo with *Agrobacterium* capable of transferring at least one gene construct thereto;
 - (c) co-cultivating the precultured embryo with *Agrobacterium* ; and
 - (d) regenerating plants expressing the gene construct.
- [c2] 2. The method of claim 1 in which after the co-cultivating, the embryo is cultured in a second medium containing a selective agent to select for embryos expressing a gene construct that confers resistance to the selective agent.
- [c3] 3. The method of claim 1 in which the MS salts are doubled and the picloram concentration is 4 mg/L.
- [c4] 4. The method of claim 1 in which the monocotyledonous plant is wheat.
- [c5] 5. A transgenic plant produced by the method of claim 1.
- [c6] 6. A method for transforming a monocotyledonous plant using an *Agrobacterium* - mediated process, comprising:
- (a) preculturing at least one immature embryo from a monocotyledonous plant in a first medium containing a concentration of glyphosate that is not sufficient to kill plant cells for a period of time sufficient to form a precultured embryo;
 - (b) contacting the precultured embryo with *Agrobacterium* capable of transferring at least one gene construct to the embryo comprising a gene that confers resistance to a glyphosate-containing herbicide;
 - (c) co-cultivating the precultured embryo with *Agrobacterium* ;
 - (d) culturing the precultured embryo in a second medium containing a concentration of glyphosate that is not sufficient to kill plant cells;

(e) culturing the embryo in a third medium containing a selective amount of glyphosate to select for embryos expressing the gene that confers resistance to a glyphosate-containing herbicide; and
(e) regenerating plants expressing the gene construct.

[c7] 7. The method of claim 6 wherein the glyphosate concentration not sufficient to kill cells is from about 0.001 to 0.5 mM

[c8] 8. The method of claim 7 wherein the glyphosate concentration is from about 0.005 to 0.05 mM.

[c9] 9. The method of claim 8 wherein the glyphosate concentration is 0.02 mM.

[c10] 10. The method of claim 6 in which the monocotyledonous plant is wheat.

[c11] 11. A transgenic plant produced by the method claim 6.

[c12] 12. A method for transforming a monocotyledonous plant using an *Agrobacterium* -mediated process, comprising:

- (a) preculturing at least one immature embryo from a monocotyledonous plant for a period of time sufficient to form a precultured embryo;
- (b) contacting the precultured embryo with *Agrobacterium* capable of transferring at least one gene construct to the embryo comprising a gene that confers resistance to a glyphosate-containing herbicide;
- (c) co-cultivating the precultured embryo with *Agrobacterium* ;
- (d) culturing the precultured embryo in a medium containing aromatic amino acids and a selective amount of glyphosate to select for embryos expressing the gene; and
- (e) regenerating plants expressing the gene construct.

[c13] 13. The method of claim 12 wherein the aromatic amino acid concentration is from about 0.001 μ M to 1 mM.

[c14] 14. The method of claim 13 wherein the aromatic amino acid concentration is from about 0.05 μ M to 0.1 mM.

[c15] 15. The method of claim 14 wherein the aromatic amino acid concentration is

from about 0.1 μ M.

[c16] 16. The method of claim 12 wherein the monocotyledonous plant is wheat.

[c17] 17. A transgenic plant produced by the method of claim 12.

[c18] 18. A method for transforming a monocotyledonous plant using an *Agrobacterium*-mediated process, comprising:
 (a) preculturing at least one immature embryo from a monocotyledonous plant for a period of time sufficient to form a precultured embryo;
 (b) contacting the precultured embryo with *Agrobacterium* capable of transferring at least one gene construct to the embryo;
 (c) co-cultivating the embryo with *Agrobacterium* ;
 (d) culturing the embryo in a first medium containing a selective agent to select for embryos expressing the gene; and
 (e) regenerating plants expressing the gene construct in a second medium containing copper.

[c19] 19. The method of claim 18 wherein the copper concentration is from about 0.001 μ M to 3 mM.

[c20] 20. The method of claim 19 wherein the copper concentration is from about 1 to 100 μ M.

[c21] 21. The method of claim 20 wherein the copper concentration is from about 2 μ M to 20 μ M.

[c22] 22. The method of claim 18 wherein the monocotyledonous plant is wheat.

[c23] 23. A transgenic plant produced by the method of claim 18.

[c24] 24. A method for transforming a monocotyledonous plant using an *Agrobacterium* -mediated process, comprising:
 (a) preculturing at least one immature embryo from a monocotyledonous plant in a first medium containing increased MS salts, increased picloram, and a concentration of glyphosate insufficient to kill plant cells for a period of time sufficient to form a precultured embryo;

- (b) contacting the precultured embryo with *Agrobacterium* capable of transferring at least one gene construct to the embryo comprising a gene that confers glyphosate resistance;
- (c) co-cultivating the embryo with *Agrobacterium* ;
- (d) culturing the embryo in a second medium containing a concentration of glyphosate insufficient to kill plant cells;
- (e) culturing the embryo in a third medium containing aromatic amino acids and a selective amount of glyphosate to select for embryos expressing the gene;
- and
- (f) regenerating plants expressing the gene in a fourth medium containing copper.

[c25] 25. The method of claim 24 wherein the plants are stably transformed.

[c26] 26. The method of claim 24 wherein the gene construct is expressed in subsequent generations.

[c27] 27. A transgenic plant produced by the method of claim 24.

[c28] 28. A method for transforming a wheat plant using an *Agrobacterium* - mediated process, comprising:

- (a) preculturing at least one immature embryo from a monocotyledonous plant in a first medium containing doubled MS salts, picloram at 4 mg/L, and glyphosate at 0.02 mM for a period of time sufficient to form a precultured embryo;
- (b) contacting the precultured embryo with *Agrobacterium* capable of transferring at least one gene construct to the embryo comprising a gene that confers glyphosate resistance;
- (c) co-cultivating the embryo with *Agrobacterium* ;
- (d) culturing the embryo in a second medium containing glyphosate at 0.02 mM;
- (e) culturing the embryo in a third medium containing aromatic amino acids at a concentration of 0.1 μ M and a selective amount of glyphosate to select for embryos expressing the gene; and
- (f) regenerating plants expressing the gene in a fourth medium containing

copper at a concentration from about 2 μ M to 20 μ M.

[c29] 29. The method of claim 28 wherein the plants are stably transformed.

[c30] 30. The method of claim 28 wherein the gene construct is expressed in subsequent generations.

[c31] 31. A transgenic plant produced by the method of claim 28.